

WHAT IS CLAIMED IS:

1. A method of obtaining a recombinant herbicide tolerance nucleic acid which can confer tolerance to an herbicide upon a plant in which the recombinant herbicide tolerance nucleic acid is present, the method comprising:
- 5 (i) recombining a plurality of variant forms of one or more parental nucleic acid, wherein the plurality of variant forms comprises segments derived from the parental nucleic acid, wherein the parental nucleic acid encodes an herbicide tolerance activity, or can be shuffled to encode an herbicide tolerance activity, and wherein the plurality of variant forms differ from each other in at least one nucleotide, to produce a library of
- 10 recombinant nucleic acids;
- (ii) screening the library to identify at least one recombinant herbicide tolerance nucleic acid, wherein the recombinant herbicide tolerance nucleic acid encodes an activity which confers herbicide tolerance to a cell.
- 15 2. The method of claim 1, wherein the recombinant herbicide tolerance nucleic acid encodes a distinct or improved herbicide tolerance activity compared to the parental nucleic acid.
- 20 3. The method of claim 1, wherein the one or more parental nucleic acid encodes an herbicide tolerance activity.
4. The method of claim 1, wherein the parental nucleic acids do not encode herbicide tolerance activity, wherein recombining the plurality of variant forms provides a
- 25 nucleic acid which encodes an herbicide tolerance activity.
5. The method of claim 1, wherein the parental nucleic acid encodes a polypeptide which is functionally or structurally similar to a herbicide target protein.
- 30 6. The method of claim 1, wherein the plurality of variant forms of the parental nucleic acid comprise allelic or interspecific variants of the parental nucleic acid.

7. The method of claim 1, wherein the plurality of variant forms of the parental nucleic acid is produced by synthesizing a plurality of nucleic acids homologous to the parental nucleic acid.

5 8. The method of claim 1, wherein the plurality of variant forms of the parental nucleic acid is produced by error-prone transcription of the parental nucleic acid or by replication of the parental nucleic acid in a mutator cell strain.

9. The method of claim 1, wherein the parental nucleic acid encodes a polypeptide or polypeptide fragment selected from the group consisting of: a P450 monooxygenase polypeptide, a glutathione sulfur transferase polypeptide, a homogluthathione sulfur transferase polypeptide, a glyphosate oxidase polypeptide, a phosphinothricin acetyl transferase polypeptide, a dichlorophenoxyacetate monooxygenase polypeptide, an acetolactate synthase polypeptide, a protoporphyrinogen oxidase polypeptide, a 5-enolpyruvylshikimate-3-phosphate synthase polypeptide, and a UDP-N-acetylglucosamine enolpyruvyltransferase polypeptide.

10. The method of claim 9, wherein the parental nucleic acid is selected from the group consisting of: a P450 monooxygenase gene from corn or wheat, a glutathione sulfur transferase gene from corn, a homogluthathione sulfur transferase gene from soybean, a glyphosate oxidase gene from a bacteria, a phosphinothricin acetyl transferase gene from a bacteria, a dichlorophenoxyacetate monooxygenase gene from a bacteria, an acetolactate synthase gene from a plant, a protoporphyrinogen oxidase gene from a plant, a protoporphyrinogen oxidase gene from an alga, an enolpyruvylshikimate-3-phosphate synthase gene from a bacteria, a enolpyruvylshikimate-3-phosphate synthase gene from a plant, and a UDP-N-acetylglucosamine enolpyruvyltransferase gene from a bacteria.

11. The method of claim 5, wherein the parental nucleic acid encodes a UDP-N-acetylglucosamine enolpyruvyltransferase, and wherein the herbicide is glyphosate.

12. The method of claim 1, wherein the library comprises a recombinant nucleic acid produced by recombining a plurality of variant forms of a parental nucleic acid selected from the group consisting of:

5 a P450 monooxygenase nucleic acid, a homoglutathione sulfur transferase nucleic acid, a glutathione sulfur transferase nucleic acid, a glyphosate oxidase nucleic acid, a phosphinothricin acetyl transferase nucleic acid, a dichlorophenoxyacetate monooxygenase nucleic acid, a acetolactate synthase nucleic acid, a enolpyruvylshikimate-3-phosphate synthase nucleic acid, and a UDP-N-acetylglucosamine enolpyruvyltransferase nucleic acid.

13. The method of claim 1, wherein the screening comprises a step selected from the group consisting of:

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- (a) screening for oxidation of the herbicide;  
(b) screening for glutathione conjugation to the herbicide or to a metabolite of the herbicide;  
(c) screening for homoglutathione conjugation to the herbicide or to a metabolite of the herbicide.

14. The method of claim 1, wherein the library of recombinant nucleic acids is present in a population of cells.

15. The method of claim 14, wherein the screening comprises growing the population of cells in or on a medium comprising the herbicide and detecting a physical difference between the herbicide and a modified form of the herbicide produced by the cells.

16. The method of claim 15, wherein the physical difference between the herbicide and the modified form of the herbicide is detected by a difference in fluorescence or absorbance between the herbicide and the modified form of the herbicide.

17. The method of claim 16, wherein the herbicide is dicamba, the recombinant herbicide tolerance nucleic acid encodes a dicamba oxidation activity, and the cells are screened for dicamba oxidation by fluorescence of an oxidized form of dicamba.

18. The method of claim 14, wherein the screening comprises growing the population of cells in or on a medium comprising the herbicide and selecting for enhanced growth of the cells in the presence of the herbicide.

5 Sub D5  
19. The method of claim 18, wherein enhanced growth of the cells requires the activity encoded by the recombinant herbicide tolerance nucleic acid.

20. The method of claim 19, wherein enhanced growth of the cells requires the product of the reaction of the herbicide by the activity encoded by the recombinant herbicide tolerance nucleic acid.

21. The method of claim 20, wherein the cell is an Mpu<sup>+</sup> strain of bacteria, the herbicide is glyphosate, and the recombinant herbicide tolerance nucleic acid encodes an activity that catalyses the conversion of glyphosate to aminomethylphosphonate.

15 Sub D6  
22. The method of claim 19, wherein the cells are an AroA<sup>-</sup> strain of bacteria, the herbicide is glyphosate, and the recombinant herbicide tolerance nucleic acid encodes an activity which catalyses the conversion of phosphoenolpyruvate plus shikimate 3-phosphate to 5-enolpyruvylshikimate-3-phosphate.

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23. The method of claim 1, the method further comprising screening the library for one or more additional activity that confers tolerance to one or more additional herbicide.

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24. The method of claim 1, wherein the step of recombining is performed in a plurality of cells.

25. The method of claim 24, further comprising:  
(a) recombining DNA from the plurality of cells that encode herbicide tolerance activity with a second library of DNA fragments, at least one of which undergoes recombination with a segment in a nucleic acid present in the cells to produce recombined cells, or recombining DNA between the plurality of cells that encode herbicide tolerance activity to produce modified cells.

26. The method of claim 25, further comprising:  
(b) recombining and screening the recombined or modified cells to produce  
further recombined cells that have evolved additionally distinct or improved herbicide  
5 tolerance activity.

27. The method of claim 26, further comprising:  
repeating (a) or (b) until the further recombined cells have acquired  
additionally distinct or improved herbicide tolerance activity.

10 28. The method of claim 1, wherein the method further comprises:  
(iii) recombining at least one recombinant herbicide tolerance nucleic acid  
with a further nucleic acid, wherein the further nucleic acid is the same or different from  
one or more of the plurality of the variant forms of (i), to produce a further library of  
15 recombinant nucleic acids;  
(iv) screening the further library to identify at least one further recombinant  
herbicide tolerance nucleic acid that encodes a further improved herbicide tolerance  
activity compared to a non-recombinant herbicide tolerance gene; and, optionally,  
repeating (iii) and (iv).

20 29. The method of claim 28, wherein the further recombinant herbicide  
tolerance nucleic acid encodes two or more distinct or improved herbicide tolerance  
activities.

25 30. The method of claim 1, wherein the library is present in bacterial cells and  
the method comprises:  
pooling multiple separate library members;  
screening the resulting pooled library members for a recombinant herbicide  
tolerance nucleic acid that encodes a distinct or improved herbicide tolerance activity  
30 compared to a non-recombinant herbicide tolerance nucleic acid; and,  
cloning the distinct or improved recombinant herbicide tolerance nucleic  
acid.

31. The method of claim 2, wherein the distinct or improved herbicide tolerance activity is selected from the group consisting of: an increase in ability to metabolize the herbicide; an increase in the range of herbicides to which the activity confers tolerance; an increase in expression level compared to that of a polypeptide encoded by the parental nucleic acid; a decrease in susceptibility to inhibition by the herbicide compared to that of an activity encoded by the parental nucleic acid; a decrease in susceptibility to protease cleavage compared to that of a polypeptide encoded by the parental nucleic acid; a decrease in susceptibility to high or low pH levels compared to that of a polypeptide encoded by the parental nucleic acid; a decrease in susceptibility to high or low temperatures compared to that of a polypeptide encoded by the parental nucleic acid; a decrease in toxicity to a host plant compared to that of a polypeptide encoded by the selected nucleic acid; and any combination of two or more thereof.

32. The method of claim 1, further comprising transducing the recombinant herbicide tolerance nucleic acid into a plant.

33. The method of claim 1, further comprising transducing the recombinant herbicide tolerance nucleic acid into a plant and testing the resulting transduced plant for tolerance to the herbicide.

34. The method of claim 1, further comprising transducing the recombinant herbicide tolerance nucleic acid into a plant and breeding the plant with a separate plant strain of the same species, followed by selection of resulting offspring for tolerance to the herbicide.

35. A library of recombinant nucleic acids made by the method of claim 1.

36. The library of claim 35, wherein the library is a phage display library.

37. A recombinant herbicide tolerance nucleic acid made by the method of claim 1.

38. A DNA shuffling mixture comprising at least three homologous DNAs, wherein each of the at least three homologous DNAs is derived from a parental nucleic acid encoding a polypeptide or polypeptide fragment selected from the group consisting of: a P450 monooxygenase, a glutathione sulfur transferase, a homoglutathione sulfur transferase, a glyphosate oxidase, a phosphinothricin acetyl transferase, a  
5 dichlorophenoxyacetate monooxygenase, an acetolactate synthase, a protoporphyrinogen oxidase, a 5-enolpyruvylshikimate-3-phosphate synthase, and a UDP-N-acetylglucosamine enolpyruvyltransferase.

10 39. The DNA shuffling mixture of claim 38, wherein the at least three homologous DNAs are present in cell culture, *in vitro*, or in a plant.

15 40. The DNA shuffling mixture of claim 38, wherein the homologous DNAs are derived from a parental nucleic acid encoding a P450 monooxygenase from corn or wheat.

20 41. The DNA shuffling mixture of claim 38, wherein at least one of the homologous DNAs is derived from a parental nucleic acid encoding a glutathione sulfur transferase from maize.

25 42. The DNA shuffling mixture of claim 38, wherein at least one of the homologous DNAs is derived from a parental nucleic acid encoding a homoglutathione sulfur transferase from soybean.

30 43. The DNA shuffling mixture of claim 38, wherein at least one of the homologous DNAs is derived from a parental nucleic acid encoding a glyphosate oxidase from a bacteria.

44. The DNA shuffling mixture of claim 38, wherein at least one of the homologous DNAs is derived from a parental nucleic acid encoding a phosphinothricin acetyl transferase from a bacteria.

45. The DNA shuffling mixture of claim 38, wherein at least one of the homologous DNAs is derived from a parental nucleic acid encoding a dichlorophenoxyacetate monooxygenase from a bacteria.

5 46. The DNA shuffling mixture of claim 38, wherein at least one of the homologous DNAs is derived from a parental nucleic acid encoding an acetolactate synthase from a plant.

10 47. The DNA shuffling mixture of claim 38, wherein at least one of the homologous DNAs is derived from a parental nucleic acid encoding a 5-enolpyruvylshikimate-3-phosphate synthase from a bacteria.

15 48. The DNA shuffling mixture of claim 38, wherein at least one of the homologous DNAs is derived from a parental nucleic acid encoding a 5-enolpyruvylshikimate-3-phosphate synthase from a plant.

20 49. The DNA shuffling mixture of claim 38, wherein at least one of the homologous DNAs is derived from a parental nucleic acid encoding a UDP-N-acetylglucosamine enolpyruvyltransferase from a bacteria.

50. The DNA shuffling mixture of claim 38, wherein at least one of the homologous DNAs is derived from a parental nucleic acid encoding a protoporphyrinogen oxidase from a plant or an alga.

25 51. A method of acquiring or improving an herbicide tolerance activity in a parental plant cell, comprising performing whole genome shuffling of a plurality of genomic nucleic acids in the plant cell, forming a modified plant cell, and screening the modified plant cell for a distinct or improved tolerance activity to one or more herbicide compared to the parental plant cell.

30 52. The method of claim 51, wherein the herbicide is selected from the group consisting of: dicamba, glyphosate, bisphosphonate, sulfentrazone, imidazolinone,



sulfonylurea, triazolopyrimidine, phenoxyacetic acid, diphenyl ether, chloroacetamide, and hydantocidin.

53. The method of claim 51, wherein the genomic nucleic acids are from a species or strain different from the parental plant cell.

54. The method of claim 51, further comprising: regenerating the modified plant cell, or a descendent cell thereof, into a plant.

55. A method of predicting long-term efficacy of a herbicide in killing a plant, the method comprising:

(i.) transforming a plurality of cells of the plant with a library of DNA fragments at least some of which undergo recombination with segments in the genome of the cells to produce modified plant cells;

(ii.) propagating the modified plant cells in a media containing the herbicide, and recovering surviving plant cells;

(iii.) recombining DNA from surviving plant cells with a further library of DNA fragments at least some of which undergo recombination with cognate segments in the DNA from the surviving plant cells to produce further modified plant cells;

(iv.) propagating further modified plant cells, in media containing the herbicide, and collecting further surviving plant cells;

(v.) repeating (iii.) and (iv.), as necessary, until a further surviving plant cell has acquired a desired degree of resistance to the herbicide, whereby the degree of resistance acquired and the number of repetitions of (iii.) and (iv.) needed to acquire it provide a measure of the long-term efficacy of the herbicide in killing the plant.

56. The method of claim 55, wherein the plant is a weed plant.

57. The method of claim 56, wherein the plant is selected from the group consisting of *Abutilon theophrasti*, *Chenopodium spp.*, *Amaranthus spp.*, *Ipomoea spp.*, *Setaria spp.*, *Echinochloa spp.*, *Solanum spp.*, *Sorghum halopense*, *Digitaria spp.*, *Panicum spp.*, *Bromus tectorum*, and *Kochia scoparia*.

58. The method of claim 55, further comprising repeatedly recombining DNA from the modified plant cells, wherein the repeated recombination is performed prior to propagating the modified plant cells in a media containing the herbicide.

5 59. The method of claim 55, further comprising dividing surviving plant cells into first and second pools, isolating the further library of DNA from the first pool and transforming the second pool with the further library.

60. The method of claim 55, wherein the further library of DNA is obtained  
10 from a species or strain different from the plant cell.